NAVAL MEDICAL RESEARCH UNIT SAN ANTONIO

SPECIAL REPORT

DEVELOPMENT OF A NOVEL ELECTROSPINNING SYSTEM WITH AUTOMATED POSITIONING AND CONTROL SOFTWARE

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Declaration of Interest

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EXECUTIVE SUMMARY

Background: Electrospinning is a nanofiber fabrication technique that has grown rapidly in recent years due to its numerous biomedical applications in wound healing and tissue engineering. The process uses an electrical charge to draw ultrafine fibers from a liquid polymer solution to form a fibrous scaffold that can support living cells or deliver drugs and small molecules to a target location. The primary components of an electrospinning system are: a dispensing needle, a high voltage power source (5 to 50 kV), a syringe pump, and a grounded collector. By adjusting the polymer composition and variables associated with the system, fiber orientations and morphologies can be precisely controlled to generate scaffolds that suit a particular application.

Objectives: To design an electrospinning system with custom software that integrates control of the primary components through a single user interface and automates the production of fiber scaffolds.

System Design: The primary hardware components were selected to accommodate a variety of electrospinning configurations, and a 3-axis gantry was built to position the dispensing needle relative to the collecting surface. Custom software was developed in LabVIEW™ to provide the operator flexibility to adjust position, dispensing, and electrical parameters dynamically and also to automate fiber generation over extended periods.

System Validation: The electrospinning system is currently operational and has successfully generated nanofibers from a variety of polymer solutions. It is capable of running for several hours and producing stable nanofiber scaffolds. The fiber diameters and morphologies of the scaffolds examined using scanning electron microscopy were found to be consistent with published results.

Conclusions: Commercial hardware components and custom software were integrated to produce an automated electrospinning system. The design of this system aimed to maximize ease of use on the part of the operator, and provide flexibility to accommodate new components and different configurations. This system will increase the throughput of fiber samples and streamline the procedure of investigating new implementations and applications.

BACKGROUND

Electrospinning is a nanofiber fabrication technique that has grown rapidly in popularity in recent years due to its potential in numerous biomedical applications. The process uses an electrical charge to draw ultrafine fibers from liquid polymer solution to form a non-woven fiber scaffold. The polymer fiber diameters can range from millimeters to as small as nanometers in scale, creating a surface area-rich material that maximizes interactions between the fibers and the biological environment [1].

The primary elements of an electrospinning system include: a dispensing needle (or spinneret), a high-voltage power supply (5 to 50 kV), a syringe pump, and a grounded collector. The polymer solution in the syringe is extruded from the spinneret at a constant rate. As high voltage is applied at the spinneret, a cone is formed at the tip. This phenomena, known as the "Taylor cone", is caused by the balance between electrostatic forces and the surface tension of the polymer. With sufficient voltage, electrostatic forces will overcome the surface tension, ejecting a thin jet of polymer from the spinneret (Figure 1). As the polymer stream travels to the collector, the solvent dries and the stream experiences whipping instabilities, which promotes the elongation and thinning of the fiber before deposition on the collecting surface. Characteristics of the resulting fiber scaffold can be controlled using a variety of parameters including: concentration and molecular weight of the polymer, voltage, flow rate, distance between the spinneret and collector, and solution properties such as viscosity and surface tension [2-4].

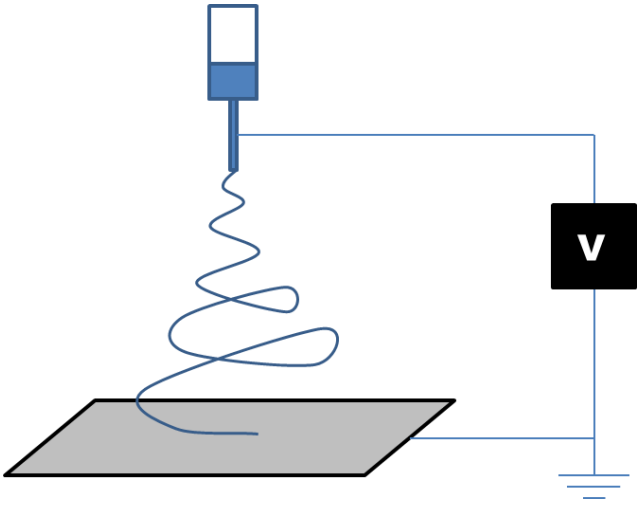

Figure 1. Electrospinning schematic. A high voltage is applied to a polymer solution dispensed from a syringe pump. Electrostatic forces stretch the droplet at the needle tip, and a thin jet of polymer is ejected from the cone, which dries and whips as it travels toward the grounded collector plate.

By modifying the general system architecture, a wide variety of fiber morphologies can be produced. For example, multiple spinnerets can be used simultaneously to create a heterogeneous scaffold comprised of different polymers, or specialized coaxial spinnerets can be used to spin one polymer type inside another. Different collectors can also be used to control fiber orientations within a scaffold. For instance, a rotating drum or patterned collecting surfaces can be used to generate aligned fibers. Electrospun nanofiber scaffolds can be engineered with a specific fiber composition, size, and orientation to support numerous applications such as drug delivery, wound dressings, filtration, and textile manufacturing [1, 5].

Investigation into biomedical applications for electrospun nanofiber scaffolds has begun only within the last few decades; there is significant room for further exploration. Challenges for the technique include optimizing the test parameters to achieve specific properties as well as producing sufficient quantities of fiber within a reasonable timeframe. The goal of this design was to automate the electrospinning process such that parameters can be set initially and the system will run for a specified duration. Using this production scheme, operators will be able to increase throughput to test spinning configurations more efficiently to suit their specific biomedical applications.

SYSTEM DESIGN

I. Hardware Overview

The electrospinning hardware consists of three sub-systems: the gantry components, the syringe pump, and the high voltage power supply. Control of the three sub-systems is achieved using a personal computer (PC) running custom software developed in LabVIEW™ (National Instruments™, Austin, TX). Figure 2 below shows a schematic of the communications between the primary components.

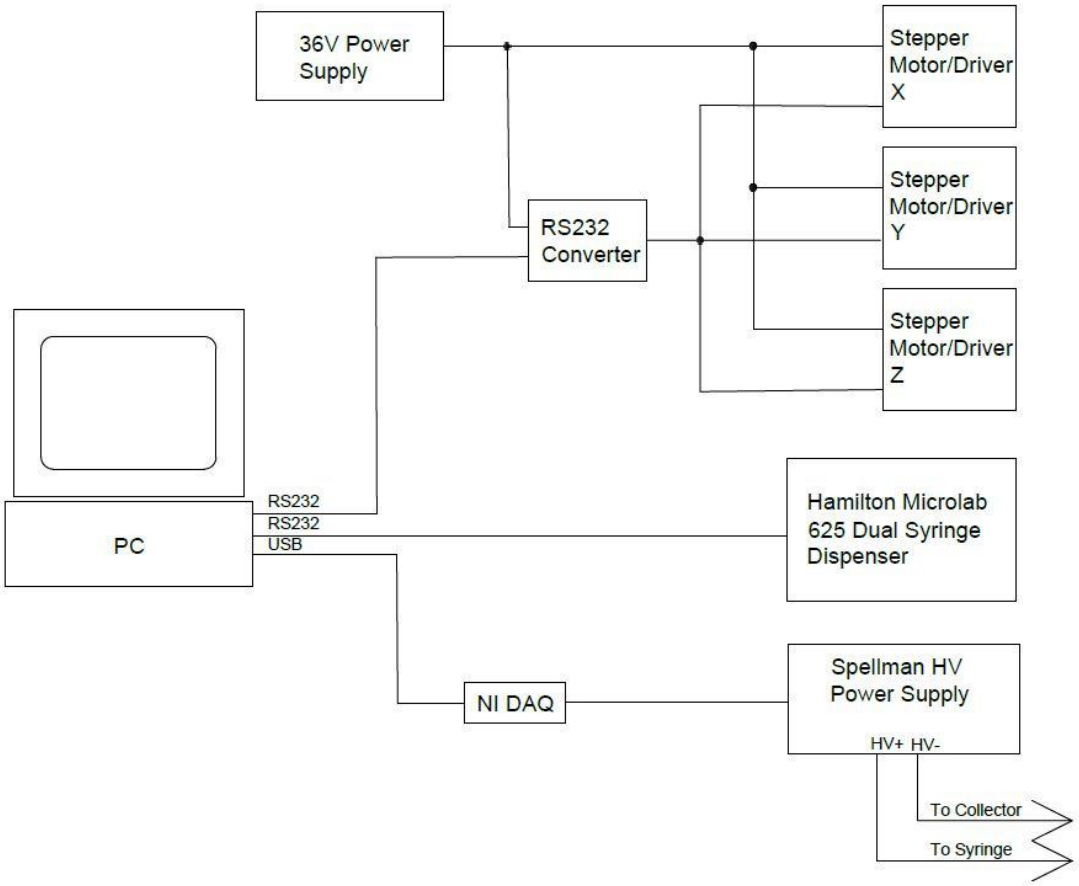

Figure 2. Electrospinner wiring diagram. The three electrospinning subsystems: gantry motors, syringe pump dispenser, and high voltage (HV) power supply, are integrated through a single PC user interface. The three gantry motors also receive power from a separate 36V power supply. The HV power supply receives its input signal from a Data Acquisition unit (DAQ) connected in line with the PC.

a. Gantry Components

Three axes of translational movement were required to adjust the position of the dispensing needle relative to the collecting surface. Each axis consists of a stepper motor that rotates a lead screw to move a plate along the track of a linear actuator. The x-axis and y-axis are coupled and move the collector plate, while an independent z-axis moves the dispensing needle.

The linear actuators (Velmex©, Bloomfield, NY, BiSlide®) have a lead screw, with a pitch of 10 turns per inch, rigidly attached to a mounting plate (Figure 3). The assembly lies inside a track enclosed on three sides. Each slide has adjustable limit switches on either end of travel to provide a soft emergency stop prior to the slide reaching a hard physical limit. The x-axis and z-axis slides have a travel distance of 15" and the y-axis slide has a travel distance of 20".

Figure 3. Linear actuator assembly. Velmex© BiSlides® (pictured with Velmex© motors) are attached orthogonally such that the base of one is mounted on top of the plate of the second. The x- and y-axes are coupled in this way in the system design, and the z-axis stands alone.

The stepper motors used in the electrospinning system (Lin Engineering© Morgan Hill, CA, Silverpak 23CE #CE-5718L-01P) each have an integrated driver and controller (Figure 4). The motors have a 1.8° full step resolution and are capable of generating microsteps as small as 1/256 of a full step. The encoder generates 1250 counts per revolution and allows the position of the gantry to be controlled and measured with a resolution of ~ 2 microns. The motor assembly is also programmable with 4kB of memory, and prewired to receive inputs from the limit switches at either end of the travel distance.

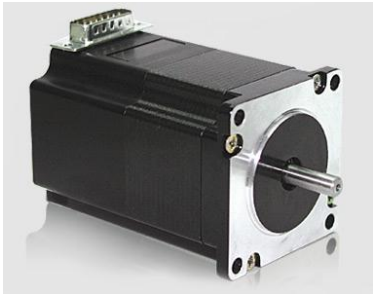

Figure 4. Motor assembly. Lin Engineering© Silverpak 23CE integrated motor, driver, and controller. The four mounting holes on the faceplate are for attachment to the end of the linear actuator assembly, with the shaft driving the lead screw, and the pin connector on top is for serial communication to the controller.

An RS232 Kit (#RS232KIT) provides a communication link between the motors and the PC (Figure 5a). The RS232 Kit converts serial communication from the PC to an indexed RS485 protocol, which allows a single PC serial port to communicate with multiple stepper motors independently. A 36V power supply (Hengfu, #HF300W-S-36) is used exclusively for the

gantry motors (Figure 5b). The power supply input is 120VAC, and it outputs 36V at 8.5A (300W).

Figure 5. a) RS232 converter kit. **b) 36V power supply.**

The gantry is enclosed in a box made of 80/20® aluminum frame with window panels made of PMMA (Figure 6). While not airtight, the box does offer some protection against contamination, and isolates the high voltage components from operator contact. The linear actuators are mounted to a small 80/20® frame inside the box that provides rigid support.

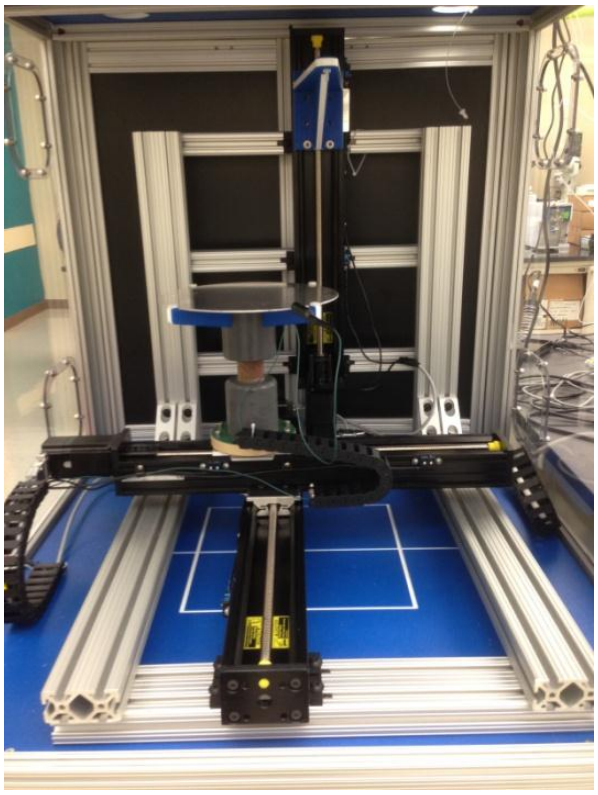

Figure 6. Electrospinner gantry components. The collector plate sits atop two coupled linear actuating slides allowing movement in the x-y plane. The dispensing needle moves in the z-dimension along a third slide. The actuators are attached to a rigid frame inside an enclosed box.

b. Syringe Pump

Polymer solution is delivered to the energized needle with a high-precision syringe pump with dual syringes (Hamilton Company®, Reno, NV, Microlab® 625, Figure 7). The instrument can be programmed from the control screen on the device to operate as a single dispenser (one solution and one syringe), dual dispenser (two solutions in two separate syringes), or continuous dispenser (two linked syringes and one solution). Because a goal of the project was to integrate the electrospinning components into a single interface, the functionality of the various syringe pump dispensing modes was replicated in LabVIEW™ and all syringe pump commands were sent via serial port.

The syringe pump uses positive displacement providing better than 99% volumetric accuracy, independent of solution viscosity, vapor pressure, and temperature, according to the manufacturer. The step resolution is 48,000 steps per stroke of the pump, independent of syringe size. The glass syringes have a conical plunger tip that extends into the valve to eliminate air, which reduces the time required to prime the lines before spinning.

Figure 7. Syringe pump. Hamilton® Microlab® 625. The left side is empty, but the right side is loaded with a glass syringe. Movement of the plunger draws in solution from the tubing entering the side of the valve, and dispenses solution out the tubing from the front of the valve.

c. High Voltage Power Supply and Grounded Collector

The high voltage power supply provides the necessary voltage differential between the needle tip and the collector plate to eject the polymer fiber from the Taylor cone (Figure 8). The

voltage required to spin fibers varies widely depending on the particular polymer and solvent combination; typical values from the literature range between 5 – 50 kV. For this system, a 60 kV power supply (Spellman® High Voltage Electronics Corporation, Hauppauge, NY, #SL60P30) was selected to support a range of polymer solutions.

Communication with the high voltage power supply was established with a data acquisition unit (National Instruments™, DAQ), which receives commands from the LabVIEW™ interface and sends a scaled control signal to the high voltage supply. The high voltage output is connected to the needle using a charged adapter disc held in place with a setscrew, and the power supply ground is connected to the collector. A second wire with an alligator clip connects the dispensing needle to ground whenever the power supply is off, to ensure the system is fully discharged when not in use. In the case of multiple spinnerets, the charged adapter disks can be connected in series to energize multiple needles.

Figure 8. High voltage power supply. Spellman®SL Series. Current and voltage controls and indicators appear on the left. On the right, a rocker switch turns power on. As a safety measure, the power supply will not output a set voltage until the red HV ON button is pressed.

II. Control Software

The general flow of the system execution is shown in Figure 9. Upon startup, the system initializes the gantry motors, syringe pump, and power supply. The gantry sends the slides to their start positions (back-left of the x-y plane, top of z-axis), stopping at the limit switches. If homing coordinates were entered, the gantry will move to the specified location in each dimension. At any point after this, the position of the collector and the gap distance between the needle and the collector can be adjusted.

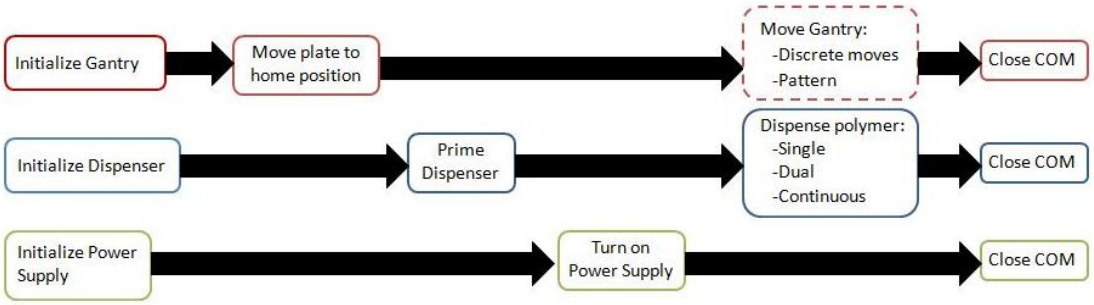

Figure 9. System flow diagram. The operator interacts with each of the components independently. Chronology flows from left to right. Dashed line indicates an optional step. Gantry and dispenser mode options are bulleted.

The polymer lines must be primed prior to the start of a spinning session. The syringe pump controls operate the same for priming as they do for dispensing during spinning. The operator enters the syringe size, draw time, and dispense time desired for the spinning configuration.

The front panel of the high voltage power supply has its own display and controls with LEDs indicating that it is powered on and ready to receive commands (Figure 10).

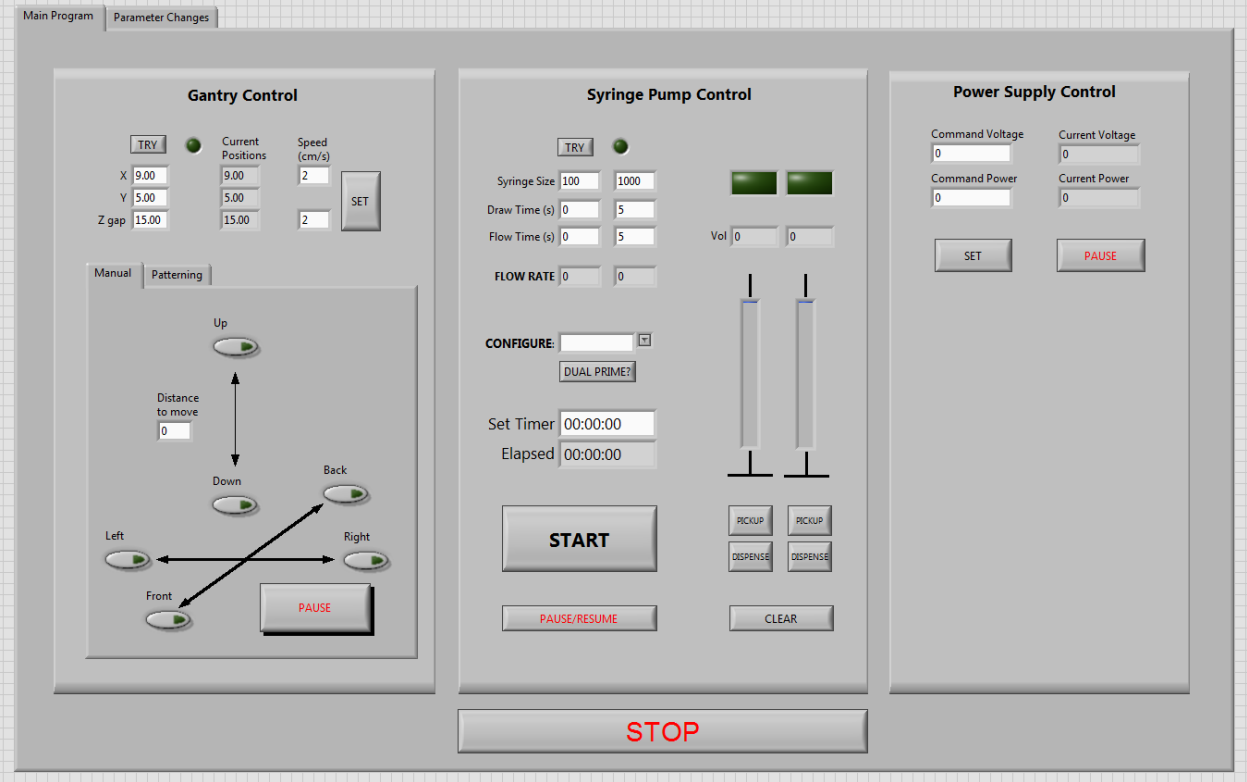

Figure 10. User Interface. Each system component has a panel column for controls and indicators.

a. Gantry Positioning

Discrete moves

At any point after initialization, the gantry can be moved in x, y or z directions. From the interface, the operator enters a distance value in the dialog box and presses the control indicating the desired direction of movement. As long as the commanded move is within the limits of the slides, the motors will move the collector. This function is intended for minor adjustments, such as fine-tuning the gap distance between the needle and collector to suit the polymer solution or positioning the needle over a specific location on the collector.

Patterning preset

Once the gantry is initialized, the operator also has the option to load a spreadsheet with patterning parameters to instruct the gantry to move along a specified path. This feature is important for creating large-area, uniform fiber scaffolds, which would not be feasible with a stationary collector.

The patterning parameters and structure were designed for flexibility. In a simple example of its application, the gantry can traverse a rectangular space by moving along one dimension, alternating direction with each row and pausing at intervals (Figure 11). The operator enters Δx and Δy distances values, which define the arbitrary coordinate grid containing the node locations of a pause or change in direction. Other input parameters include speed and time to run, which is linked to the syringe pump. Once the gantry has reached the last node specified in the routine, the program executes the path in reverse. The collector plate will run this forward and reverse sequence continuously until stopped by the operator or at the end of a pre-defined period of time.

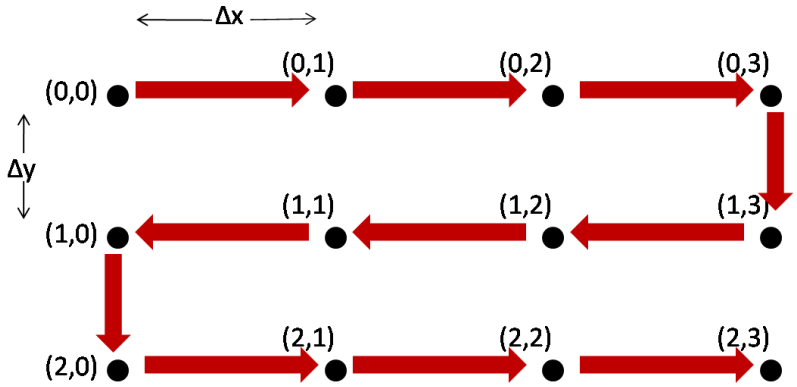

Figure 11. Sample trajectory for patterned spinning. The operator specifies the gap distances (Δx, Δy), speed, pause time (if desired), and coordinates for locations that require a pause or a change in direction.

b. Syringe Pump

Parameters

The controls for the dispensing pump are *Syringe Size*, *Draw Time*, and *Flow Time*, each for the left and right sides. The syringe size value scales the current position of the syringe pump to display an accurate volume in real time. The draw and dispense rates are controlled by a measure of time because speed commands must be sent to the dispenser in seconds per full stroke of the syringe. This measure requires an integer value for the number of seconds. For the operator's convenience, there is an indicator that displays the resulting flow rate from the operator-defined parameters. Draw time and flow time are controlled independently, which is important for very viscous solutions that need a longer draw time to minimize air bubbles but can dispense at a faster rate.

Similar to the gantry, the dispenser can also perform a discrete action. This is useful when the syringe is mid-stroke prior to the start of a run and the operator would like it to be full for maximum continuity. A discrete move can also be used to empty a partially-filled syringe. When the pickup or dispense control is pressed, the syringe pump will move to the corresponding end of its stroke (bottom for pickup, top for dispense) at the rate corresponding to the draw time or flow time specified in the numeric controls entered previously. If the move needs to be halted before the dispenser reaches the end of its stroke, the operator can pause, and then later resume or clear the buffer to remove the command from memory.

There is a timer located on the syringe pump panel if the operator would like the system to run for a specified amount of time and then stop automatically. An indicator displays the elapsed time since dispensing began, regardless of whether a *Stop Time* has been set.

Dispense Modes

In ***Single*** dispense mode, one side of the syringe pump is in use, with the inlet on the side of the valve and the outlet on the front. If not already in the filled position, the syringe to be used makes a full draw of the polymer solution. Once started, the syringe begins to dispense at the flow rate specified. If a timer is set, the dispenser will run until time has run out; otherwise, the

syringe will draw and dispense until the operator manually pauses the dispenser. This mode is sufficient if spinning continuity is not essential.

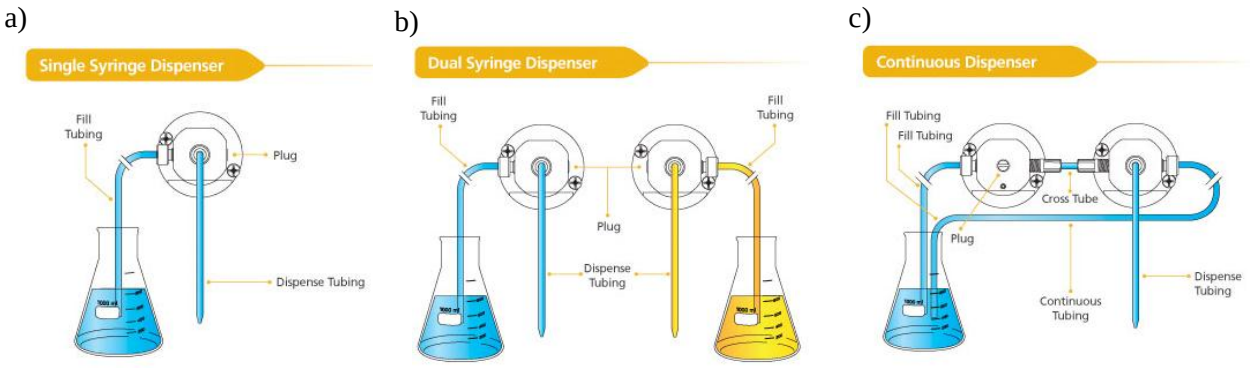

Figure 12. Syringe pump valve and tube configurations [5]. a) Single: one syringe. b) Double: two independent syringes with different solutions. c) Continuous: two syringes with the same solution. Valves are connected with a cross tube; alternating, one syringe draws while the other dispenses.

The ***Dual*** dispense mode is used for spinning two syringes of polymer solutions. The syringe size, flow rate, and draw rate can be set separately. If two polymers are dispensed at different rates, the controller can set both syringes to refill when the first syringe becomes empty to maximize continuity of the flow. Alternatively, the two sides can operate independently, completely filling and dispensing at their respective rates. This feature supports modified electrospinning configurations, including coaxial, where the needle tip dispenses a hollow tube of one polymer surrounding an inner core of another, and multiple spinnerets, which can be used to increase throughput.

Continuous dispense is used for uninterrupted dispensing of a single polymer and requires two syringes of the same size and a bridge piece to connect the two valves. Both syringes have an input line from the solution, but one syringe's output line goes to the bridge connecting the other syringe and the other syringe's output line goes to the spinneret. In this configuration, one syringe is always picking up while the other is dispensing. The syringes reach opposing limits of travel at the same time, the valves turn over, and the process repeats. This feature is important in patterning configurations when the collector is moving and scaffold uniformity depends upon consistent fiber deposition.

c. High Voltage Power Supply

The LabVIEW™ interface controls the output of the high voltage power supply using a data acquisition unit (National Instruments™, DAQ). The DAQ communicates the desired set point using a 0-10V analog signal, which is proportional to the 0-60kV high voltage power supply output. Similarly, the high voltage power supply generates a 0-10V signal proportional to the actual high voltage output, which is read by the DAQ. This allows any error to be monitored and ensures the actual voltage being generated corresponds with the desired set point. Voltage can be changed at any time, and commands from the PC interface can be overridden with the on/off buttons on the front of the device.

SYSTEM VALIDATION

The electrospinning system has been in operation and has produced fiber scaffolds from a number of different spinning configurations and polymer/solvent solutions. As validation of system functionality, images were taken using scanning electron microscopy (SEM) of the scaffolds. Figure 13a is a 4% weight per unit volume polyethylene oxide (PEO) solution (molecular weight 300 kDa) in a 1:1 acetone (ACE) to dichloromethane (DCM) solution. The spinning parameters were: 15 kV, 1 mL/h flow rate, and a 15 cm gap distance between the spinneret tip and collector.

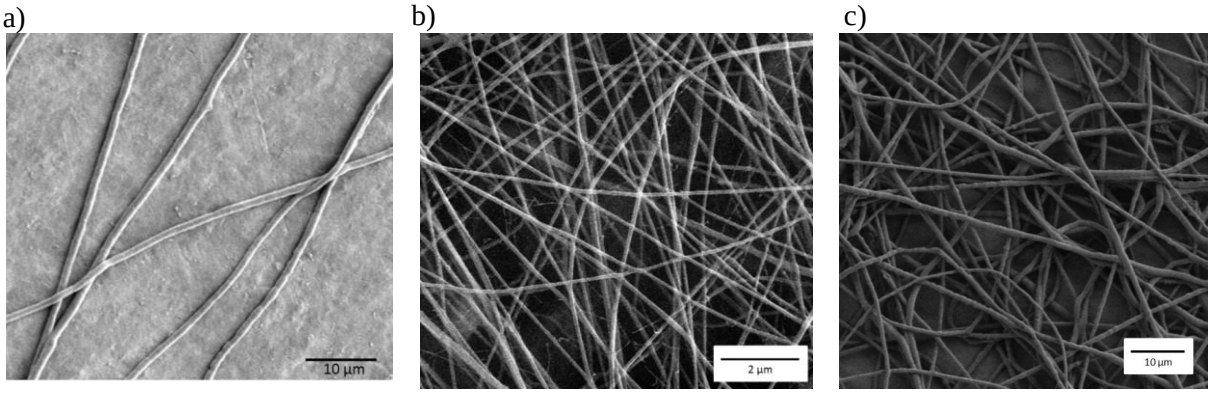

Figure 13. Example SEM images of the electrospun fibers. The composition and spinning parameters of voltage, flow rate, and gap distance between the needle tip and collector were: a) PEO in ACE and DCM at 15 kV, 1 mL/h, 15 cm; b) CS and PEO in acetic acid at 12 kV, 0.3 uL/h, 15 cm; c) PLGA in ACE at 8 kV, 1 mL/h, 10 cm.

In Figure 13b, the polymer was a 2:1 chitosan (CS) to PEO in 45% acetic acid. Spinning took place at 12 kV, 0.3 uL/h flow rate, and a gap distance of 15 cm. Lastly, Figure 13c shows a 10% solution of 85:15 poly(lactic-co-glycolic acid) (PLGA, molecular weight 250 kDa) in ACE. The parameters this time were 8 kV, 1 mL/h flow rate, and a 10 cm gap distance.

The SEM images showed that fibers spun with the developed system possessed the desired morphologies, comparable to scaffolds found in the literature. The fibers are uniform in diameter, and virtually free of beads. These images indicate that all the critical parameters were optimized.

FUTURE DEVELOPMENT

The electrospinning system developed successfully generates polymer fiber scaffolds through a user-friendly and flexible interface. The apparatus can accommodate a wide variety of polymer solutions to produce fibers of varying size and composition, and scaffolds of varying thickness and area. Future development efforts will focus on further automating the system and adding the ability to control fiber orientations.

During initial testing of the system, temperature and humidity were found to significantly affect the success or failure of a set of configuration parameters. As these variables fluctuated, the voltage and the separation distance between the spinneret and the collector had to be adjusted to spin fibers successfully. System controls will be added to maintain temperature and humidity inside the enclosure, reducing the amount of variability between different days of operation and reducing the amount of adjustment required by the operator. Modifications will allow configuration parameters to be standardized for a given polymer solution and ensure consistency and reproducibility of fibers.

Another area for development is the configuration of the collector. Depending on the specific application of the scaffold, the spinning setup can be modified to achieve different fiber orientations. For example, there are several variations of collector for producing aligned fibers. Some configurations utilize a rotating drum which aligns the depositing fibers along its circumference (Figure 14a, b). Another arrangement has parallel rod electrodes positioned such that the fibers span the gap in an aligned fashion (Figure 14c). These setups, however, possess their own challenges and limitations. For instance, thicker scaffolds are not possible with some

rotating drum configurations, and fiber breakage and degree of alignment are potential issues if the flow rate is not properly synchronized with the speed of rotation [6].

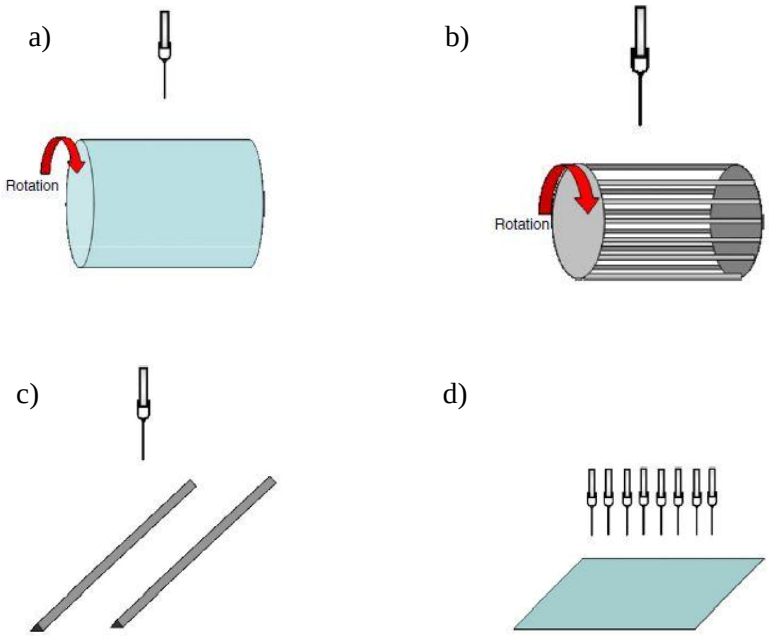

Figure 14. Alternative collector configurations from Teo, 2006. a) Rotating drum. b) Rotating drum wire collector. c) Parallel electrodes. d) Multiple spinnerets.

Preliminary testing has been conducted to create coaxial fibers, and further testing will expand the technique by testing new polymer combinations with different spinning parameters. Coaxial spinning could also be used in conjunction with one of the collector setups for aligned fibers outlined above. In addition, adding multiple spinnerets (Figure 14d) to any of the collector setups would be a simple means to reduce spinning durations and thereby increase throughput [7].

A long-term application for this electrospinning system involves the development a fiber scaffold loaded with antibiotics onto an implant surface to reduce the risk of infection. This capability would require the gantry to translate in three dimensions as well as rotate the implant such that the gap distance and angle from the spinneret is kept constant and the surface is evenly coated. These factors would be critical in maximizing adhesion of the fiber scaffold. Implementation would involve importing a 3D model of the implant to calculate spatial points on the surface. This technology could potentially allow for patient-specific implants that utilize nanofiber scaffolds to minimize infection and promote integration of tissues.

CONCLUSION

Commercial hardware components and custom software were integrated to produce an automated electrospinning system. The combined interface for a three-axis gantry, syringe pump, and high voltage power supply enables the operator to modify the spinning parameters from a single display. Adjustments can be made on-the-fly, which limits stoppage in spinning, and the system can also be set to generate fibers over an extended duration with minimal supervision.

The software design aimed to maximize ease-of-use. The control software is reconfigurable and flexible to accept new hardware components and different hardware arrangements. Because the system automates dispensing of the polymer and positioning of the collector, operators can allocate less time to monitoring the electrospinning process. This functionality will enable a higher throughput of fiber samples and streamline the investigation of new configurations and biomedical applications for the technique.

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14. ABSTRACT

Electrospinning is a nanofiber fabrication technique that has grown rapidly in popularity in recent years due to its potential in numerous biomedical applications in the military. The objective is to design an electrospinning system with custom software to control through a single interface and to automate the electrospinning spinning process. Using this production scheme, operators will be able to increase throughput to test spinning configurations more efficiently to suit their specific biomedical applications. The electrospinning system has been in operation and can successfully generate nanofibers from a variety of polymer solutions. It is capable of running for several hours and producing stable nanofiber scaffolds. The design of this system aimed to maximize ease of use on the part of the operator, and provide flexibility to accommodate new components and different configurations. This system will increase the throughput of fiber samples and streamline the procedure of investigating new implementations and applications.

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